Use of diethyl squarate for the coupling of oligosaccharide amines to carrier proteins and characterization of the resulting neoglycoproteins by MALDI-TOF mass spectrometry.

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The 8-methoxycarbonyloctyl glycosides of GlcNAc beta, Gal beta 1-4Glc beta, Fuc alpha 1-2Fuc alpha 1-3GalNAc beta and Fuc alpha 1-2Gal beta 1-3[Fuc alpha 1-4]GlcNac beta were converted to primary amines by reaction with neat ethylenediamine and then coupled to bovine serum albumin (BSA) using diethyl squarate as the connector. The average degree of incorporation of the sugar onto the protein, as well as the molecular weight distribution, could be conveniently determined using matrix assisted laser desorption ionization/time of flight (MALDI-TOF) mass spectrometry thus avoiding cumbersome structure-dependent colour-tests or analysis of cleaved ligand. The present coupling method has the advantages of proceeding under very mild conditions, yielding controlled incorporation values and can reliably be used for the coupling of very small amounts (mg) of oligosaccharide.

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Coupling of acid labile Salmonella specific oligosaccharides to macromolecular carriers.

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A coupling method for covalent attachment of acid labile oligosaccharides isolated from S. typhimurium O-polysaccharide to macromolecular carriers is described. Arylamine groups were introduced into the terminal reducing end of oligosaccharides by reacting them with 2-(4-aminophenyl)-ethylamine. After subsequent conversion to the corresponding saccharide-phenylisothiocyanato derivatives saccharides were covalently linked to free epsilon-lysylamine groups of different carrier proteins. The resulting conjugates were highly immunogenic and elicited in rabbits both anti-harptenic and anti-carrier protein specific antibodies. Some of the advantages of this coupling procedure are: (i) it can be used with oligosaccharides containing highly acid or alkali labile structures and/or glycosidic linkages, (ii) it produces conjugates with high degrees of substitution at low saccharide/protein molar input ratios, (iii) it does not grossly affect the immunogenic specificities of the carrier protein, and (iv) it is suitable for preparation of highly substituted affinity columns, e.g., coupling to a polyacrylamide matrix.

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